THE EYES OF THE BLIND VERTEBRATES OF NORTH AMERICA. III.

THE STRUCTURE AND ONTOGENIC DEGENERATION OF THE EYES OF THE MISSOURI CAVE SALAMANDER, AN ACCOUNT BASED ON MATERIAL COLLECTED WITH A GRANT FROM THE ELIZABETH THOMPSON SCIENCE FUND.[1]

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A SINGLE specimen of a salamander was discovered in Rock House Cave, Barrie County, Missouri, by Mr. F. A. Sampson in July, 1891. The specimen was described by Stejneger in the *Proceedings of the U. S. National Museum*, Vol. XV, p. 115, as Typhlotriton spelæus. His diagnosis reads as follows: "Vertebrae opistocœlous; parasphenoid teeth; vomerine teeth; eyes concealed under the continuous skin of the head; tongue attached in front and along the median line, free laterally and posteriorly; maxillar and mandibular teeth small and numerous; vomerine teeth in two strongly curved series; parasphenoid patches separate; nostrils very small; toes five; sixteen costal grooves, or eighteen if counting the axillary and groin grooves; tail slightly compressed, not finned; toes nearly half webbed; vomerine teeth in two *V*-shaped series with the curvatures directed forward; gular fold strong, very concave anteriorly; color uniformly pale."

Stejneger fully appreciated the value and nature of his discovery. He says: "Although many of our salamanders are known to inhabit caves, this seems to be the only one, so far discovered, which, like some of the other animals exclusively living in caves, has become blind or nearly so." This was written by him before he discovered the Typhlomolge in the underground streams of Texas.

[1] *Contribution from the Zoölogical Laboratory of the Indiana University, No. 31.*

A preliminary note by the present authors (*Proc. Ind. Acad. Sci., 1898*, p. 252, 1899) completes the list of papers dealing with this species.

In the spring of 1897 Dr. Eigenmann visited Rock House Cave and secured a number of larvae, which Dr. Stejneger pronounced the larvae of Typhlotriton. Later Mr. E. A. Schultze informed him that he had seen this salamander in the underground passage to Blondi's Throne Room in Marble Cave, Stone County, Missouri. In September of 1898 he visited this cave and secured four adults and three larvae of Typhlotriton. A large number of the larvae were obtained from Rock House Cave a few days later. Those from the latter cave were found under loose stones and gravel in the rivulet at the mouth of the cave. They had been exposed to the light. It is scarcely supposable that those from Marble Cave had ever been affected by the light. In the caves both larvae and adults are found under the stones, the old ones in and out of the water. Occasionally one is seen lying on the bottom of a pool.

In the aquarium the larvae creep into or under anything available; a glass tube serves as a "hiding" place. The rubber tube admitting water to the aquarium is sometimes occupied by several during a temporary cessation of the flow of water. A wire screen sloping from the bottom of the aquarium formed the most popular collecting place for the larvae. They collected beneath this, although it was no protection from the light. From these observations it seems probable that stereotropism rather than negative heliotropism accounts for the presence of this species in the caves, and that this reaction has been retained after the long stay of the species in caves necessary to account for the changes in its eyes.

The eyes of the larvae when examined from the surface appear perfectly normal, but they are little used in distinguishing objects. When hungry they will strike at a stick held in the hand as they would at food. A stick lying at the bottom of the aquarium undisturbed is not molested. They strike at a worm when touched by it, or when it approaches close enough for its motion to be perceived.

In the larvae up to 90 mm. long the skin passes over the

eye without forming a free orbital rim and the eye does not protrude beyond the general contour of the head. In the adult from 97 mm. on, the eye forms a bead-like projection. There are in the adult distinct lids. These are closed over the eye, covering it entirely, the slit being much too small for the eye. The lower lid is free from pigment, but the upper lid, which closes over the lower, is as thickly pigmented as any other part of the body.

Stejneger says of the eyes that they are "small, only slightly raised, and covered by the continuous skin of the head, with only a shallow groove to indicate the opening between the lids, the underlying eyes visible as two ill-defined dusky spots."

In sections the lids are seen to overlap one another some distance, forming an obscure, free orbital rim. Fig. 1, *a*, is a median section of the lids and corneal epithelium of an eye .954 mm. in diameter, taken from an adult specimen 106 mm. in length. In this section the upper lid overlaps the lower lid .216 mm., or more than one-fifth the diameter of the eye. Passing from the median section toward the corners of the eye, the lower lid unites with the underlying tissue first. When observed from the top the upper lid covers the entire eye. The orbital slit is .17 mm. in length. The conjunctival pocket extends some distance forward and backward beyond the slit. The eye increases in size but little from the larval to the adult stage, and its growth is not proportional to the growth in length of the animal. (See comparative measurements of the eyes at the close of the paper.)

The following is a series of measurements on the larvae of Typhlotriton.

	Rock House Cave.	Rock House Cave.	Marble Cave.
Specimen . . .	54 mm. long.	78 mm. long.	88 mm. long.
Size of pupil . .	.432 mm.	.640 mm.	———
Length of eye . .	1.30 mm.	1.50 mm.	1.60 mm.
From optic nerve to front of lens .	.80 mm.	1.20 mm.	———
Vertical diameter	———	1.248 mm.	1.28 mm.

Sections of the adult and larva from Marble Cave were made in the usual manner. The six normal eye muscles were pres-

ent in Typhlotriton. The m. recti form a sheath about the optic nerve in its distal part and spread out from it near the eye. In the adult the sclera is a layer of uniform thickness except in the region of the entrance of the optic nerve. It is not usually separated from the adjoining parts of the eye, but in places is retracted a short distance from the choroid coat by the action of reagents. It is for the most part fibrous, with few compressed nuclei, and varies from 18 to 40μ in thickness. In the larva a narrow cartilaginous band surrounds all but the ventral wall of the eye. In a specimen 35 mm. long the width of the band is about 30μ, its thickness 16μ. In three adult specimens the sclera of only one had any traces of cartilage. In the right eye of the adult specimen 103 mm. long a cartilage about 36μ thick, 60μ wide, and not more than 40μ long is found on the upper face of the eye. The absence of this cartilage in the adult has probably no connection with the degeneration of the eye. Its presence is probably a larval characteristic which disappears as the gills disappear during the metamorphosis.

The average thickness of the cornea is 40μ. In the adult it is covered by a layer of stratified epithelium, 25μ in thickness, consisting of three rows of cells. The cells of the inner row are columnar in shape, those of the middle row rounded, and those of the outer row are very much flattened and elongated (Fig. 1, *a*).

In the adult the choroid coat is usually separated from the pigment layer, but adheres closely to the sclera. In general it is thicker at the back part of the eye, and quite decidedly so at the entrance of the optic nerve. The lens is normal. Its size is given in the table at the end of the paper.

The layers of the retina are well developed in the larva. The retina of the larva differs from that of an Amblystoma larva in the greater thickness of its ganglionic layer. This layer is, in the young larva of Typhlotriton, composed of five or six layers of cells. This thickness may in part be an artifact, since the retinae examined are shrunken away from the pigment epithelium, and the ganglionic layer is in contact with the lens. In the larva 90 mm. long this layer has been reduced

to not more than three series of cells. Aside from the differences noted above, the eye of the larvae of Typhlotriton is apparently normal in all of its histological details. This relative thickness in the different sizes of the larvae may be gathered from Figs. 2–5 and from the comparative table at the end of the paper.

Figs. 2–5 are drawn with the same magnification and show the relative thickness of the different layers in the retinae of the larvae of different sizes and of the adult. The adult retina is reduced in thickness by the absence of the rods and cones and the (partial ?) atrophy of the outer reticular layer and by the thinning of the ganglionic layer. The ganglionic layer in the adult contains from two to five rows of cells. In this respect, the adult approaches the condition found in Amblystoma more than the young does. The inner reticular layer is comparatively thick, that of the young being thicker than that of the adult.

In the adult the inner nuclear layer is continuous with the outer nuclear layer. (See Fig. 5.)

The inner nuclear layer consists of about seven series of cells in the smallest larva and of four to seven in the largest. The cells in the preparations available cannot be separated into bipolar and spongioblastic layers, nor are horizontal cell layers distinguishable. The outer reticular layer is well differentiated but quite thin in the larvae, and is irregular in outline, adapting itself to the overlying nuclei which encroach on its outlines. In the adult this layer is indistinguishable by the same methods that make it conspicuous in the larva. In places there appeared an open space where the outer reticular layer should be (Fig. 9), but none of its structure remains. It is fair to suppose that the fibers forming this layer are resorbed during the metamorphosis. This layer seems to be the very first obliterated by the processes of degeneration both ontogenic and phylogenic in this as in other vertebrates with a degenerating eye.

The greatest change during and shortly after metamorphosis takes place in the layer of the rods and cones. In the larva 35 mm. long, from the mouth of Rock House Cave, the rods reach an extreme length of 50 μ. The relative sizes and

number of these as compared with the much smaller cones may be gathered from Fig. 2, *a*.

In the larva 90 mm. long the outer segments of the rods are much shorter and stain less conspicuously than in the younger. The nuclei of the outer nuclear layer are distinctly in two layers, whereas in the younger they are in three less regular layers. The cones are correspondingly fainter than in the young. It is surprising that whereas in the larva 90 mm. long we find the rods and cones well developed they have greatly degenerated or practically disappeared in the adult only a few mm. longer. In an adult specimen 97 mm. long the rods have retained their normal shape and position, but I have not been able to detect any differentiation into inner and outer segments. In longer ones most of the nuclei of the outer series have become rounded at both ends. But one cone was found in eyes of the adult over 100 mm. long. It is shown in Fig. 6. In an adult specimen 103 mm. long filmy rods are still evident. They appear as conical spaces above the nuclei free from pigment rather than as possessing any demonstrable structure. Just at the margin of the place where the pigment has been torn from the retina one of these is drawn out to a great length. The pigment in this individual extends in places down between the cells of the cones. This latter condition appears in a very exaggerated form in the eye of Typhlomolge. In tangential section this condition and the filmy rods give rise to the appearance represented in Fig. 5, *a*.

Distinct signs of ontogenic degeneration are also seen in other parts of the retina. For instance, many nuclei of the inner series of the outer nuclear layer are shriveled. In some eyes the ganglionic nuclei have for the greater part lost their granular structure and show a homogeneous pasty condition, only a few cells with granular nuclei being present (Fig. 5). The same is true in large part of the inner nuclei of the inner nuclear layer. This condition of the ganglionic nuclei is not entirely confined to the adult but is also found in the larva.

Some of the modifications in the shapes of the outer nuclei in the adult are shown in the figures. In Fig. 7 the upper

portion of the nucleus is very much elongated. This form is of frequent occurrence. In Fig. 8 is shown the common form where the nuclei are simple elliptical bodies, which give no evidence whatever of any processes uniting them with the other elements of the retina. The Müllerian fibers are profusely present and of very large size in both larva and adult. In both adult and young the optic nerve enters as a single strand and passes entirely through the layers. A heavy mass of pigment is found following the optic nerve to within a short distance of the brain.

AVERAGE MEASUREMENTS OF THE EYES OF TYPHLOTRITON.

LENGTH OF SPECIMEN.	35 mm.	48 mm.	62 mm.	90 mm.	97 mm.	103 mm.	106 mm.
Vertical diameter of eye	810	800	—	960	—	800	1170
From front of lens to back of eye .	600	672	—	720	720	720	1134
Outer nuclear layer with the rods .	76	42	112	36	28	28	—
Outer reticular layer	1	2	—	—	—	—	—
Inner nuclear layer	76	72	80	50	48	72	72
Inner reticular layer	16	20	16	24	8	8	13
Ganglionic layer	68	56	64	32	24	26	26
Pigment layer	4	16	—	—	8	20	22
Optic nerve	20	25	—	—	—	23	29
Lens	342	300	—	500	432	430	504

SUMMARY.

Typhlotriton is an incipient blind salamander living in the caves of southwestern Missouri. It detects its food by the sense of touch without the use of its eyes. It is stereotropic. Its eyes show the early stages in the steps of degeneration from those of salamanders living in the open to those of the degenerate Typhlomolge from the caves of Texas. The lids are in process of obliteration, the upper overlapping the lower so that the eye is always covered in the adult. The sclera possesses a cartilaginous band in the larval stages but not in the adult. The disappearance of the cartilage is probably an incident of the metamorphosis, not of the degeneration the eye is undergoing. The lens is normal. The retina is normal in

the larva with a proportionally thicker ganglionic layer than in the related epigæan forms. Marked ontogenic degenerations take place during and shortly after the metamorphosis. *a.* The outer reticular layer disappears. *b.* The rods and cones lose their complexity of structure, such as differentiation into inner and outer segments, and finally are lost altogether.

EXPLANATION OF FIGURES.

All drawings were made with the aid of the Abbe camera from sectioned balsam preparations. The comparative measurements (p. 39) furnish the key to the magnification:

ps.	palpabra superior.	*pi.*	palpabra inferior.
1.	pigment epithelium.	2.	rods and cones.
3.	outer nuclear layer.	4.	outer reticular layer.
5.	horizontal cell layer.	6.	inner nuclear layer.
7.	spongioblastic layer.	8.	inner reticular layer.
9.	ganglionic layer.	10.	optic fibers.

Fig. 1. Diagrammatic representation of the eye drawn to scale.

Fig. 1, *a*. Vertical section through the cornea and lids of an adult.

Fig. 2. Section of the retina, exclusive of pigment cells, of a larva 35 mm. long.

Fig. 2, *a*. Tangential section through the rods and cones about on a level with the innermost extent of the pigment which is seen on the right, showing the relative sizes and abundance of the rods and cones.

Fig. 3. Section of the retina of a larva 48 mm. long.

Fig. 4. Section of the retina of a larva 90 mm. long.

Fig. 4, *a*. Tangential section showing the rods and cones at about the inner limit of the pigment which is seen on the left.

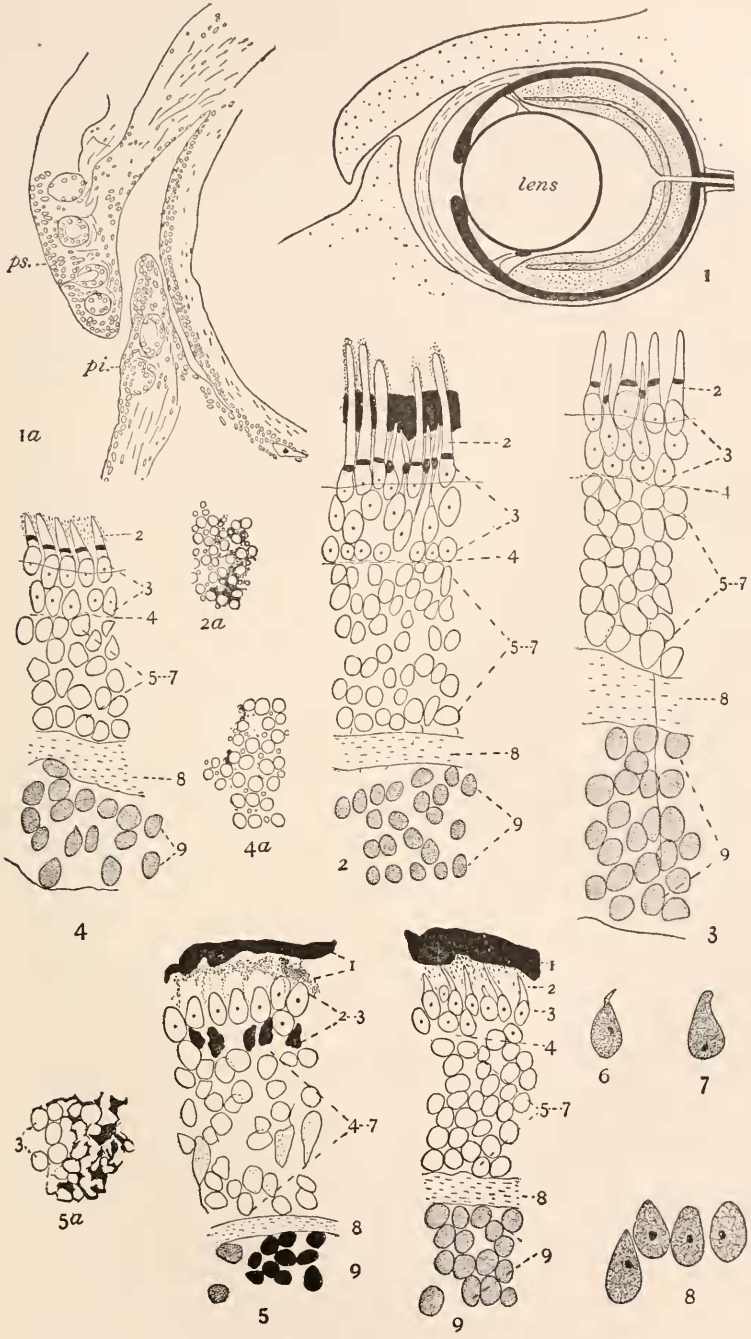
Fig. 5. Section of the retina of an adult 106 mm. long.

Fig. 5, *a*. Tangential section at about the inner limit of the pigment.

Fig. 6. The only cone found in the eyes of adults.

Fig. 7, 8. Difference in the shape of the outermost series of cells in the outer nuclear layer.

Fig. 9. Section of the retina of an adult 97 mm. long.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